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Original Article

Age Susceptibility of Wistar Rats to Alloxan-Induced Diabetes: A Paradox

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Abstract

Selection of appropriate ages of rats for experimental diabetes mellitus studies has been posing great challenges to researchers as the rats may either not come down with diabetes or may even die upon induction. This study investigated the influence of age as a possible factor affecting successful induction of diabetes mellitus in albino Wistar rats. One hundred male albino Wistar rats of varying ages weighing between 23.63 ± 0.46 g and 279.00 ± 27.00 g and assigned into ten (10) groups of ten rats per group were used for the study. Diabetes was induced in groups 1-9 consisting of rats aged 3, 5, 7, 9, 11, 13, 15, 17 and 19 weeks respectively by injecting 160 mg/kg of alloxan monohydrate intraperitoneally while group 10 rats (aged 12 weeks) served as normal control. Fasting blood glucose levels and the weights of the rats were determined on 0 h, 24 h, 48 h, 72 h and 96 h post alloxan monohydrate injection. Body conditions and behaviors of the rats were subjectively scored. Results showed that rats in groups 3-9 came down with hyperglycemia 48 h post-alloxan injection. The body conditions and behaviors of groups 3 (aged 7 weeks) and 4 (Aged 9 weeks) rats compared very well ($p<0.05$) with those of the normal control rats. It was concluded that rats aged 7-9 weeks were most suitable for use in studies involving induction of experimental diabetes mellitus using alloxan monohydrate at the dose of 160 mg/kg intraperitoneally.

Keywords: animals; diabetogens; hyperglycemia; influence; length of time

Introduction

Diabetes mellitus is an important metabolic disease affecting both man and animal all over the world (Dewanjee *et al*., 2008). Two major types of diabetes mellitus are traditionally distinguished. Type 1 diabetes mellitus results from auto-immune destruction of the beta cells while type 2 is occasioned by insulin resistance followed by inability of beta cells to compensate for more insulin production (WHO, 1999). While type 1 is modeled by chemical ablation of the pancreatic beta cells, type 2 is tailored in obese animals with some degrees of insulin resistance (King, 2012).

Experimental diabetes mellitus may be induced by chemical or surgical methods (Etuk, 2010). Some of the chemicals used in inducing experimental diabetes include alloxan monohydrate and streptozotocin (Szkudelski *et al*., 1998). Researchers have proposed different mechanisms involved in the successful induction of experimental diabetes by these chemicals. It is in literature that alloxan monohydrate induces diabetes by destroying the pancreatic beta cells of the islet of langerhans through cascade of mechanisms involving redox pathways (Lenzen *et al*., 1996; Lenzen, 2008). This will result to reduction in insulin production thereby leading to accumulation of glucose beyond normal values in the blood (hyperglycemia). Similar mechanism of action has also been proposed for streptozotocin (Srinivasan and Ramarao, 2007).

Rodents such as rats and mice are among the animal models that have been used consistently in studies involving diabetes mellitus (Srinivasan and Ramarao, 2007). There are a lot of genetically manipulated rodents for diabetes studies (Etuk, 2010). Albino Wistar rats are very common laboratory rats for diabetes studies. They are outbred of albino rats with long ears, wide head and a tail length which is not as long as the body length. Wistar rats are more active than their Sprague dawley counterpart (Krinke, 2000).

Irregular observations of diabetes induction failures and different levels of success in induction in Wistar rats using alloxan monohydrate at the same dose in our laboratory constitute the driving force for this study. Some researchers submitted that successful induction of diabetes using alloxan depends on the dose of alloxan, animal species, route of administration and nutritional status of the animal (Federiuk, 2004).

There is dearth of information on the effect of age of Wistar rats on the success of diabetes induction using alloxan monohydrate. This study was therefore designed to investigate the influence of age as a possible factor affecting successful induction of diabetes in Wistar rats.

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Materials and Methods

Animals

One hundred (100) male albino Wistar rats of varying ages were obtained and bred in litters from the laboratory animal house of Veterinary Medicine Department, University of Nigeria, Nsukka Enugu State, Nigeria. All the rats were fed with Vital® grower (Benin, Nigeria) feed and clean water *ad libitum*. They were housed in a standard stainless-steel cage. The experimental protocol used in this study was approved by the Ethics Committee of the University of Nigeria, Nsukka and conforms with guide to the care and use of animals in research and teaching of University of Nigeria, Nsukka, Enugu state, Nigeria (ECUN/174290).

Experimental design

The one hundred (100) male Wistar rats were assigned to ten (10) groups of ten (10) rats per group based on their ages. Groups 1-9 consist of rats aged 3 weeks, 5 weeks, 7 weeks, 9 weeks, 11 weeks, 13 weeks, 15 weeks, 17 weeks and 19 weeks respectively while rat's in-group 10 were aged 12 weeks. Diabetes mellitus was induced in rats of groups 1-9 by single intraperitoneal injection of alloxan monohydrate (Sigma Aldrich, UK) at the dose of 160 mg/kg (Venugopal *et al*., 1998) while rats in-group 10 served as normal control. The fasting blood glucose levels were determined before the injection of the alloxan monohydrate and subsequently 24 h, 48 h, 72 h and 96 h post alloxan monohydrate injection. The blood samples were obtained by tail snip. The weights of the rats were equally determined alongside with the fasting blood glucose values with the aid of electronic weighing balance (Mettler, Germany).

Induction of experimental diabetes mellitus

The method of Venugopal *et al*. (1998) was used. The rats in groups 1-9 were fasted (feed alone was withdrawn) for 16 h (5 pm to 8 am) prior to diabetes induction. Induction was done by single intraperitoneal injection of 160 mg/kg of alloxan monohydrate. The fasting blood glucose levels were then monitored after 24 h, 48 h and 72 h (By 8 am daily) post induction. The rats were fasted for 16 h each time determination of fasting blood glucose (FBG) value was made. Rats with fasting blood glucose values greater than 126 mg/dl were considered diabetic (WHO, 1999).

Preparation of alloxan monohydrate

Alloxan monohydrate was prepared by dissolving a calculated quantity of the powder in a measured quantity of distilled water. The concentration of the alloxan monohydrate was determined by using the formular below guided by OECD's (Organisation of Economic Corporation and Development's) procedures (Erhirhie *et al*., 2014):

Concentration (mg/ml) = Weight of the Rat (kg) × Dose of Alloxan monohydrate (mg/kg) / Volume of alloxan monohydrate administered (ml). The quantity of alloxan monohydrate given to the highest weighed rat was fixed at 1 ml to enable determination of the appropriate concentration for the study. Injection of alloxan monohydrate to the rats was done immediately upon reconstitution. Administration of alloxan monohydrate was done following the formula below:

Volume (ml) = Weight of rat (kg) × Dose of Alloxan monohydrate (mg/kg) / Concentration of alloxan monohydrate (mg/ml).

Determination of weight

The electronic weighing balance was adjusted to zero with beaker before the determination of the weights of the rats. Thereafter, rats were put each in a beaker and placed on the weighing balance where the reading was obtained digitally.

Statistical analysis

The data obtained were analyzed using One-way Analysis of Variance (ANOVA) with statistical package for social sciences (SPSS) version 20. Duncan's Multiple Range post hoc test was used to separate variant means. Probability values greater or equal to 0.05 ($p \leq 0.05$) were considered significant. The results were presented in tables as Mean (± Standard Error of Mean).

Results

The FBG of all the rats in groups 3-9 were lower compared to their corresponding pre-induction values 24 h post alloxan injection. There were significant ($p<0.05$) increases in the FBG 48 h post induction compared to the pre-induction values. The increases persisted till the duration of the experiment. The FBG of the rats in group 10 (Control group) remained statistically similar until the duration of the experiment. Rats in groups 1(aged 3 weeks) and 2 (aged 5 weeks) died 24 h post alloxan injection (Table 1).

Results indicated that the percentage increases in FBG levels of all the rats in all the groups were significantly ($p<0.05$) higher than those of the rats in-group 10 (Control group) across the duration of the experiment. Rats in groups 6-9 had significantly higher FBG compared to others 24 h post induction. However, 96 h post induction, the percentage change in FBG levels of rats in-group 4 (9 weeks) were significantly ($p<0.05$) higher than those of the other groups. The changes in the FBG of all the rats in groups 3-9 were statistically similar ($p>0.05$) 48 h and 72 h post induction (Table 2).

The body weight of the diabetic rats was significantly ($p<0.05$) reduced 96 h post induction when compared to their corresponding pre-induction values in most of the induced groups (Groups 3-8). However, the body weights of all the rats in groups 9 (19 weeks) and 10 (control) remained statistically similar ($p>0.05$) till the end of the experiment (Table 3).

The percentage decreases in the body weight of the diabetic rats were statistically the same 96 h post induction. Significant percentage ($p<0.05$) decreases in the body weights were observed in group 4 rats (Aged 9 weeks) 24 h-72 h post induction compared to the decreases in the weights of other diabetic rat groups. Percentage increases in the body weight of non-diabetic rats (Group 10) were recorded (Table 4).

The body condition score was done according to Hickman and Swan (2010). The general body condition and behaviour of rats in groups 3 (aged 7 weeks) and 4 (aged 9 weeks) compared very well ($p>0.05$) with those of the rats in group 10 (Non-diabetic control) while those of the rats in groups 6-9 were statistically the same ($p>0.05$) and were better compared to the body condition of the rats in group 5 (aged 11 weeks) (Table 5).

Table 1. Fasting blood glucose (FBG) levels of alloxan-induced diabetic rats of varying ages monitored for 96 h

Group	Age (Weeks)	Fasting blood glucose levels (mg/dl) post induction				
		Preinduction	24 h	48 h	72 h	96 h
One	3	90.33 ± 3.33	-	-	-	-
Two	5	89.33 ± 5.48	-	-	-	-
Three	7	96.00 ± 0.57^{a}	60.00 ± 2.88^{a}	466.33 ± 13.30^{b}	566.00 ± 34.00^{b}	599.33 ± 0.33^{b}
Four	9	89.66 ± 1.45^{a}	65.66 ± 2.84^{a}	490.00 ± 100.00^{b}	528.33 ± 16.75^{b}	598.66 ± 0.33^{b}
Five	11	95.66 ± 1.85^{a}	55.33 ± 2.40^{a}	584.33 ± 12.01^{b}	544.33 ± 37.87^{b}	599.33 ± 0.57^{b}
Six	13	95.33 ± 3.17^{b}	41.00 ± 1.52^{a}	576.00 ± 23.50^{c}	599.33 ± 0.33^{c}	600.00 ± 0.57^{c}
Seven	15	94.66 ± 2.40^{b}	33.66 ± 1.85^{a}	589.00 ± 10.50^{c}	599.66 ± 0.66^{c}	600.00 ±0.00^{c}
Eight	17	94.66 ± 1.20^{b}	34.33 ± 0.88^{a}	589.00 ± 10.50^{c}	599.00 ± 0.57^{c}	600.00 ± 0.00^{c}
Nine	19	98.00 ± 0.57^{b}	42.33 ± 1.70^{a}	553.66 ± 45.83^{c}	600.00 ± 0.57^{c}	599.66 ± 0.33^{c}
Ten	12	94.00 ± 6.02^{a}	95.00 ± 2.00^{a}	101.00 ± 6.08^{a}	101.00 ± 5.50^{a}	99.33 ±0.88^{a}

Different superscripts a,b,c across the same row indicate significant difference at $p<0.05$

Table 2. Percentage changes in FBG levels of alloxan-induced diabetic rats of varying ages monitored for 96 h

Group	Age (Weeks)	Percentage change in fasting blood glucose levels post induction			
		24 h	48 h	72 h	96 h
Three	7	-37.00 ± 2.88^{b}	383.33 ± 139.20^{b}	489.00 ± 35.69^{b}	523.66 ± 4.05^{b}
Four	9	-26.66 ± 2.33^{c}	443.00 ± 105.00^{b}	489.66 ± 28.4^{b}	567.66 ± 10.83^{c}
Five	11	-41.33 ± 3.71^{b}	511.00 ± 20.99^{b}	469.33 ± 44.24^{b}	526.33 ± 11.92^{b}
Six	13	-56.33 ± 5.50^{a}	506.00 ± 38.97^{b}	530.00 ± 21.59^{b}	530.00 ± 21.59^{bc}
Seven	15	-64.00 ± 2.08^{a}	522.00 ± 5.50^{b}	534.00 ± 16.80^{b}	534.00 ± 16.80^{bc}
Eight	17	-63.00 ± 0.57^{a}	521.00 ± 7.76^{b}	532.66 ± 7.68^{b}	533.00 ± 8.18^{bc}
Nine	19	-56.33 ± 1.85^{a}	531.00 ± 22.59^{b}	512.00 ± 4.04^{b}	511.66 ± 3.75^{b}
Ten	12	10.00 ± 4.16^{d}	7.00 ±2.00^{a}	7.33 ± 3.28^{a}	8.00 ± 6.00^{a}

Different superscripts a,b, c, d along the same column indicate significant difference at $p<0.05$

Table 3. Body weight of alloxan-induced diabetic rats of varying ages monitored for 96 h

Group	Age (Weeks)	Body weight (g)				
		Preinduction	24 h	48 h	72 h	96 h
One	3	23.63 ± 0.46	-	-	-	-
Two	5	28.50 ± 2.32	-	-	-	-
Three	7	67.50 ± 1.70^{c}	61.96 ± 0.92^{b}	59.26 ± 0.64^{ab}	57.68 ± 0.33^{a}	56.76 ± 0.88^{a}
Four	9	90.40 ± 1.18^{c}	76.66 ± 0.74^{b}	72.86 ± 1.39^{a}	71.33 ± 1.20^{a}	70.00 ± 1.01^{a}
Five	11	125.63 ± 4.52^{b}	112.33 ± 5.36^{ab}	107.33 ± 6.35^{a}	107.66 ± 3.71^{a}	105.33 ± 2.96^{a}
Six	13	155.56 ± 3.24^{b}	151.33 ± 1.85^{b}	149.33 ± 1.85^{b}	139.00 ± 0.57^{b}	120.00 ± 2.88^{a}
Seven	15	189.00 ± 8.62^{b}	191.00 ± 0.57^{b}	186.66 ± 3.52^{b}	180.33 ± 0.88^{b}	160.33 ±18.70^{a}
Eight	17	251.30 ± 11.57^{b}	241.00 ± 1.52^{ab}	239.66 ± 0.66^{ab}	230.66 ± 6.35^{ab}	212.66 ± 18.88^{a}
Nine	19	276.36 ± 31.66^{a}	279.00 ± 27.00^{a}	271.33 ± 24.37^{a}	255.33 ± 28.52^{a}	272.33 ± 13.83^{a}
Ten	12	146.03 ± 7.06$_{a}$	147.00 ± 7.23^{a}	157.00 ± 2.51^{a}	158.66 ± 2.33^{a}	163.33 ±14.52^{a}

Different superscripts a,b,c across the same row indicate significant difference at $p<0.05$

Table 4. Percentage changes in body weight of alloxan-induced diabetic rats of varying ages monitored for 96 h

Group	Age (Weeks)	Percentage change in body weight			
		24 h	48 h	72 h	96 h
Three	7	-7.66± 1.20^{bc}	-11.66 ± 2.18^{ab}	-14.00 ± 2.00^{ab}	-15.33 ± 1.20^{a}
Four	9	-14.66 ± 2.02^{a}	-19.00 ± 1.52^{a}	-20.33 ± 1.66^{a}	-22.02 ± 2.08^{a}
Five	11	-10.00 ± 1.52^{b}	-15.33 ± 6.17^{b}	-13.66 ± 6.35^{b}	-15.66 ± 1.70^{a}
Six	13	-2.33 ± 1.85^{cd}	-3.67 ± 2.33^{b}	-10.00 ± 2.08^{b}	-22.66 ± 2.18^{a}
Seven	15	-6.00 ± 2.64^{bcd}	-6.33 ± 1.20^{b}	-6.66 ± 1.76^{b}	-15.66 ± 11.25^{a}
Eight	17	-4.67 ± 3.70^{bcd}	-4.66 ± 3.66^{b}	-7.33 ± 2.72^{b}	-15.00 ± 3.50^{a}
Nine	19	1.33 ± 1.33^{cd}	-3.67 ± 2.02^{b}	-7.33 ± 1.33^{b}	-12.66 ± 4.66^{a}
Ten	12	0.003 ± 0.003^{c}	7.67 ±6.22^{c}	9.00 ± 6.65^{c}	11.66 ± 8.74^{b}

Different superscripts a,b, c along the same column indicate significant difference at $p<0.05$

Table 5. Assessment of body and behavioral condition of alloxan-induced diabetic rats monitored for 96 h

Group	Age	Condition
One	3	0.00 ± 0.00^{a}
Two	5	0.00 ± 0.00^{a}
Three	7	3.75 ± 0.25^{d}
Four	9	3.75 ± 0.25^{d}
Five	11	2.00 ± 0.40^{b}
Six	13	2.75 ± 0.25^{c}
Seven	15	3.00 ± 0.00^{c}
Eight	17	3.00 ± 0.00^{c}
Nine	19	3.25 ± 0.25^{cd}
Ten	12	4.00 ± 0.00^{d}

Different superscripts a,b, c, d along the same column indicate significant difference at $p<0.05$
0=Dead, 1= Moribund, 2= Weak and dull, 3= Weak and alert, 4= Agile and alert

Discussion

The study investigated possible influence of age of albino Wistar rats in the successful induction of experimental diabetes mellitus using alloxan monohydrate.

Twenty-four (24 h) hours post injection of alloxan monohydrate, the FBG levels of the induced rats (Groups 1-9) were appreciably lower than their corresponding pre-induction values (Table 1). This may be consequent upon the effect of alloxan monohydrate on the pancreatic islet cells. Alloxan monohydrate has destructive effects on the islet cells of the pancreas. Researchers have reported that hypoglycemia is the first stage of alloxan-induced hyperglycemia (Lenzen *et al*., 1996; Lenzen, 2008). Influx of alloxan monohydrate via GLUT2 transporter into the pancreatic beta cell increases calcium ion concentration that destroys the beta cells and triggers overproduction of insulin that in turn leads to hypoglycemia (Kliber *et al*., 1996). Subsequently, the FBG of the rats in groups 3-9 increased significantly ($p<0.05$) 48 h through 96 h post alloxan injection (Table 1). Earlier researchers had reported hyperglycemia 48 h following alloxan injections (Tasaka *et al*., 1988; Lenzen, 2008). This may have resulted following exhaustion of insulin secretion by the glucose analogue, alloxan monohydrate (Kliber *et al*., 1996), thus leading to hyperinsulinemia and the attendant hyperglycemic episode. It is believed that within 24 h post-alloxan monohydrate administration, most of the beta cells of the islet of langerhans have been destroyed leading to release of more insulin into the circulation. This explains why there is hypoglycemia within this period. However, after 24 h, insulin levels in the circulation begin to wane since the beta cells that secrete them have been appreciably destroyed thus leading to a gradual buildup of glucose in the blood (hyperglycemia). Rats in groups 1 (aged 3 weeks) and 2 (Aged 5 weeks) could not survive the initial hypoglycemic episode beyond 24 h. We unarguably attributed the death of these groups of rats to hypoglycemia because at the point of death, their blood glucose levels were so low. This may be attributed either to the immature pancreas or due to overdose of the chemical (Alloxan monohydrate). We also opine that the tested dose (160 mg/kg) of alloxan administered may have also been responsible for the death. This goes a long way to also caution against the use of up to that dose in relatively immature rats like these ones. Researchers reported that the dose of alloxan in rats should be between 40-200 mg/kg depending on the route of administration and the nutritional status of the animal (Szkudelski *et al*., 1998). They equally noted that alloxan has a narrow diabetogenic dose and that overdose could lead to death. Researchers have reported death of experimental goat following alloxan administration (Haghdoost *et al*., 2007).

The higher percentage increases in the FBG of all the diabetic groups compared to the non-diabetic normal control was attributed to the effect of alloxan monohydrate. Alloxan monohydrate is known to evoke a sudden rise in circulating insulin levels thereby leading to a corresponding decrease in the sugar levels (Lachin and Reza, 2012). Insulin is a pancreatic hormone involved in glucose metabolism. It literally 'drags' the circulating glucose into cells thereby reducing the plasma glucose levels. The glucose transporters are dependent on the insulin signaling. The body weights of the alloxanized rats were significantly ($p<0.05$) reduced when compared to their corresponding pre-induction counterparts. This may have resulted from the effect of diabetes. Unexplained weight loss is a cardinal sign of diabetes mellitus (Cook and Plotnick, 2008). This unexplained weight loss however may be attributed to the fact that abnormal metabolism of carbohydrate, protein and fat accompany diabetes mellitus.

Observation of better body and behavioural conditions in the rats of 7-9 weeks old albino Wistar rats despite the high fasting glucose values when compared to the other older diabetic rats (aged 11-19 weeks) is paradoxical. However, it may be that at this age, the rats were better equipped to handle (metabolize) the drug, alloxan compared to the older rats. Alloxan has been reported to infiltrate other organs such as liver, kidney, brain following parenteral administration (Lee *et al*., 2010). The liver in particular is responsible for detoxification and biotransformation of xenobiotics. Probably the functional capacities of these organs (liver particularly) are at optimum at this age (7-9 weeks old rats) making them able to handle some deleterious effects due to alloxan administration. It is a well-known phenomenon that alloxan generates a lot of reactive oxygen species (ROS) which have oxidative and degenerative effects on body cells (Lachin and Reza, 2012; Rohilla and Ali, 2012). The body and behavioural conditions of 7-9 weeks rats compared very well to that of the normal control group.

Conclusions

From the results of the experiment, it was concluded that while all the albino Wistar rats aged 7-19 weeks can be used for experimental diabetes induction, those aged 7-9 weeks appeared to be better candidates. Albino Wistar rats aged 3-5 weeks should not be alloxanized at 160 mg/kg for the purposes of induction of experimental diabetes as this could lead to massive mortality of the rats. Further studies could be performed to determine the appropriate dose of alloxan that can be used to induce diabetes in albino wistar rats aged 3-5 weeks.

Conflicts of interest

The authors declare that there are no conflicts of interest related to this article.

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